Journal of Cardiology (2010) **56**, 59—65

ScienceDirect

journal homepage: www.elsevier.com/locate/jjcc

Original article

A novel cardiac myosin-binding protein C S297X mutation in hypertrophic cardiomyopathy

Takayoshi Hirota (MD), Toru Kubo (MD), Hiroaki Kitaoka (MD, FJCC)*, Tomoyuki Hamada (MD), Yuichi Baba (MD), Kayo Hayato (MD), Makoto Okawa (MD), Naohito Yamasaki (MD), Yoshihisa Matsumura (MD, FJCC), Toshikazu Yabe (MD), Yoshinori L. Doi (MD, FJCC)

Department of Medicine and Geriatrics, Kochi Medical School, Oko-cho, Nankoku-shi, Kochi 783-8505, Japan

Received 25 September 2009; received in revised form 8 January 2010; accepted 12 February 2010
Available online 23 March 2010

KEYWORDS
Hypertrophic cardiomyopathy;
Cardiac myosin-binding protein C gene;
Nonsense mutation

Summary
Background: Mutations in the cardiac myosin-binding protein C gene (*MYBPC3*) have been reported to be associated with delayed expression of hypertrophic cardiomyopathy (HCM) and a relatively good prognosis.
Purpose: The aim of this study was to evaluate clinical manifestations in patients with familial HCM caused by a novel nonsense mutation, S297X, in *MYBPC3*.
Methods: We analyzed the sarcomere protein genes in 93 probands with HCM.
Results: The nonsense mutation S297X in *MYBPC3* was present in nine subjects from two unrelated families. Eight of those nine subjects with this mutation were found to be phenotype-positive and the remaining individual was not affected phenotypically. The age range at diagnosis was 9—75 years. There was no family history of sudden death in either family. At presentation, there were various left ventricular hypertrophy (LVH) patterns, including Maron type III hypertrophy from the LV base to apex, hypertrophy confined to the anterolateral wall at the basal LV wall. Two patients showed a significant LV outflow tract gradient and one patient showed intra-right-ventricular obstruction. During follow-up, one patient was repeatedly hospitalized for the treatment of heart failure after development of paroxysmal atrial fibrillation at the age of 86 years and the remaining eight subjects were in relatively stable condition and did not require hospitalization for the treatment of HCM-related events.
Conclusion: The novel mutation S297X in *MYBPC3* causes HCM in a broad range of ages and heterogeneous clinical manifestations, though the clinical course in patients with this mutation seems to be benign.

* Corresponding author. Tel.: +81 88 880 2352; fax: +81 88 880 2349.
E-mail address: kitaokah@kochi-u.ac.jp (H. Kitaoka).

0914-5087/$ — see front matter
doi:10.1016/j.jjcc.2010.02.004

Introduction

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disorder, clinically defined as thickening of the myocardial wall in the absence of any other cause for left ventricular hypertrophy (LVH) [1—4]. Molecular genetic studies have revealed that HCM is caused by mutations in more than 10 genes that encode sarcomere contractile proteins [5—7]. Results of preliminary genetic studies on HCM have suggested that particular gene abnormalities were related to specific clinical phenotypes such as degree of hypertrophy, risk of sudden death, onset time of the disease, and penetrance in families. For example, patients with β-myosin heavy chain gene (*MYH7*) mutations tend to have severe disease of early onset [8,9]. Cardiac troponin T gene (*TNNT2*) mutations are generally associated with a high incidence of sudden death despite only mild LVH [10,11]. On the other hand, mutations in the cardiac myosin-binding protein C gene (*MYBPC3*) have been reported to be associated with delayed expression of hypertrophy and a relatively good prognosis [12—16].

The purpose of this study was to evaluate clinical manifestations in patients with familial HCM caused by a novel nonsense mutation, S297X, in *MYBPC3*.

Methods

Subjects

The subjects were 93 probands with familial or sporadic HCM who were seen at Kochi Medical School Hospital. The diagnosis of HCM was based on echocardiographic demonstration of an unexplained LVH, i.e., maximum LV wall thickness $\geq$ 15 mm. Following identification of the S297X mutation in *MYBPC3*, pedigree analysis, including both clinical evaluation and genotyping, was performed. Informed consent was obtained from all subjects or their parents in accordance with the guidelines of the Ethics Committee on Medical Research of Kochi Medical School.

Clinical evaluations

Evaluation of probands and relatives included medical history, clinical examination, 12-lead electrocardiography (ECG), M-mode, two-dimensional (2D) and Doppler echocardiography, and ambulatory 24-h Holter ECG analysis. The severity and distribution of LVH were assessed in the parasternal short axis plane at mitral valve and papillary muscle levels [17,18]. Maximum LV wall thickness was defined as the greatest thickness in any single segment. Left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured from M-mode and 2D images obtained from parasternal long-axis views, and fractional shortening ($FS = 100 \times ((LVEDD - LVESD)/LVEDD)$) was calculated. LV outflow tract gradient was calculated from continuous-wave Doppler using the simplified Bernoulli equation. Right ventricular wall thickness was evaluated in the parasternal long-axis view, and a wall thickness of more than 5 mm was defined as hypertrophy.

Phenotype-positive was defined by the following criteria for relatives: (1) maximum LV wall thickness $\geq$ 13 mm; (2) presence of major abnormalities on ECG (i.e., Q wave $\geq$ 0.04 s in duration or one-fourth of the ensuing R wave in depth in at least two leads, significant ST-T changes, and Romhilt-Estes score > 4); or (3) a combination of criteria 1 and 2.

Data regarding survival and clinical status of patients were collected during serial clinic visits. Evaluation of the phenotype was completed before the determination of the genotype.

Genetic analysis

Peripheral blood samples were taken at the time of clinical evaluation, and they were frozen and stored at −20 °C. Deoxyribonucleic acid (DNA) was extracted using a DNA purification kit from QIAGEN Inc. (no. 51104; Hilden, Germany). In vitro amplification of genomic DNA was performed using the polymerase chain reaction (PCR). Information on primer sequences and PCR conditions is available upon request. Sequencing was performed using a BigDye Terminator Cycle Sequencing Kit from Applied Biosystems Inc. (no. 4336774; Foster City, CA, USA). The sequences were analyzed on an ABI PRISM 3100-Avant Genetic Analyzer in accordance with the manual of the manufacturer. In patients in whom the mutation was identified, confirmation was obtained by re-analysis with direct sequencing from a second blood sample. Mutation analysis was carried out for the 5 most common sarcomere protein gene abnormalities: *MYBPC3*, *MYH7*, *TNNT2*, cardiac troponin I (*TNNI3*), and alpha-tropomyosin (*TPM1*) genes.

Results

Genetic results

Fourteen mutations were identified in 28 probands: S297X, P106fs, H379P, V593fs, G805S, R945fs in *MYBPC3*, R243C, N562K, R663C, R869C, E1049D, T1928 M in *MYH7*, D46 V in *TNNT2*, and R162W in *TNNI3* [19,20]. The nonsense mutation S297X in *MYBPC3* (a C-to-G transition in exon 9 replacing a serine residue with a termination signal) was identified in two of the 93 HCM probands. Relatives of two probands, totaling 11 members, were studied further. Genetic analysis revealed that a total of nine subjects, including two probands, had an S297X mutation (Fig. 1). This mutation was thought to be disease-causing based on presence of the mutation in all affected individuals and absence of sequence variation in at least 200 chromosomes from healthy individuals.

Clinical manifestations

The clinical characteristics of the nine subjects at presentation are summarized in Table 1. Eight of those nine patients with an S297X mutation were found to be phenotype-positive: seven patients with echocardiographic evidence of increased LV wall thickness and one patient (H087-III-2) with only ECG abnormality (maximum LV wall thickness

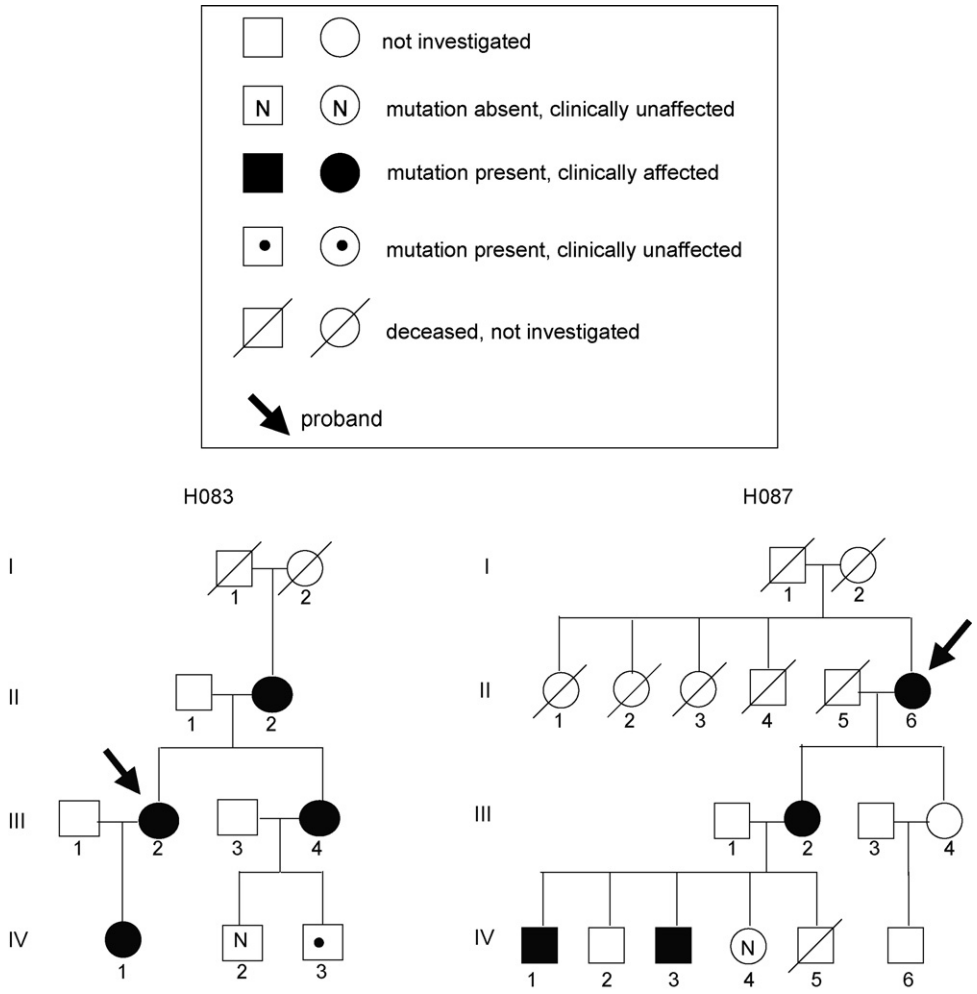

Figure 1 Pedigree of families H083 and H087. The genotypic status and phenotypic status of subjects are indicated.

of 11 mm). The remaining individual (H083-IV-3) was not affected phenotypically (age at last evaluation: 12 years). Of the eight phenotype-positive patients, three patients were evaluated because of symptoms, one patient because of ECG abnormality, and four patients because of family screening. The age range at diagnosis was 9—75 years. One patient (H083-IV-1) showed definite LV hypertrophy on echocardiography (maximum LV wall thickness: 19 mm) without any symptoms at 9 years of age. None of those patients showed ventricular tachycardia. There was no family history of sudden death in either family. Table 2 shows the echocardiographic characteristics of the nine subjects with this mutation at initial evaluation. At presentation, there were various LVH patterns, although many patients showed Maron type III hypertrophy. Two patients showed a significant LV outflow tract gradient (pressure gradient at rest $\geq$ 30 mmHg) and one patient showed intra-right-ventricular obstruction (gradient: 13 mmHg). Right ventricular hypertrophy was seen in three patients.

Table 3 shows the clinical courses of the nine subjects with this mutation. One patient (H087-II-6) was repeatedly hospitalized for the treatment of heart failure after a development of paroxysmal atrial fibrillation, although LV systolic function was preserved at the last follow-up (Fig. 2). She had normal coronary angiography. The remaining eight subjects were in relatively stable condition and did not require hospitalization for the treatment of HCM-related events. One patient (H083-IV-1), who was diagnosed as having HCM at 9 years of age, had progression of increased LV wall thickness (19—25 mm over a period of 3 years).

Discussion

This is a first report on detailed clinical presentations of patients with a novel mutation, S297X, in *MYBPC3* [19]. The results in this study provide an additional insight into the clinical manifestations of *MYBPC3* gene abnormalities.

To date, more than 200 different mutations in different sarcomere genes have been reported in patients with HCM [5—7]. Nonsense mutations were found much less frequently than missense mutations in sarcomere genes, and most of them were reported to be in *MYBPC3* [21,22]. An S297X mutation is thought to result in a truncation of the protein, including loss of C-terminal myosin and titin binding sites [5,23].

This novel nonsense mutation causes HCM in a broad range of ages. One patient (H083-IV-1) was diagnosed as

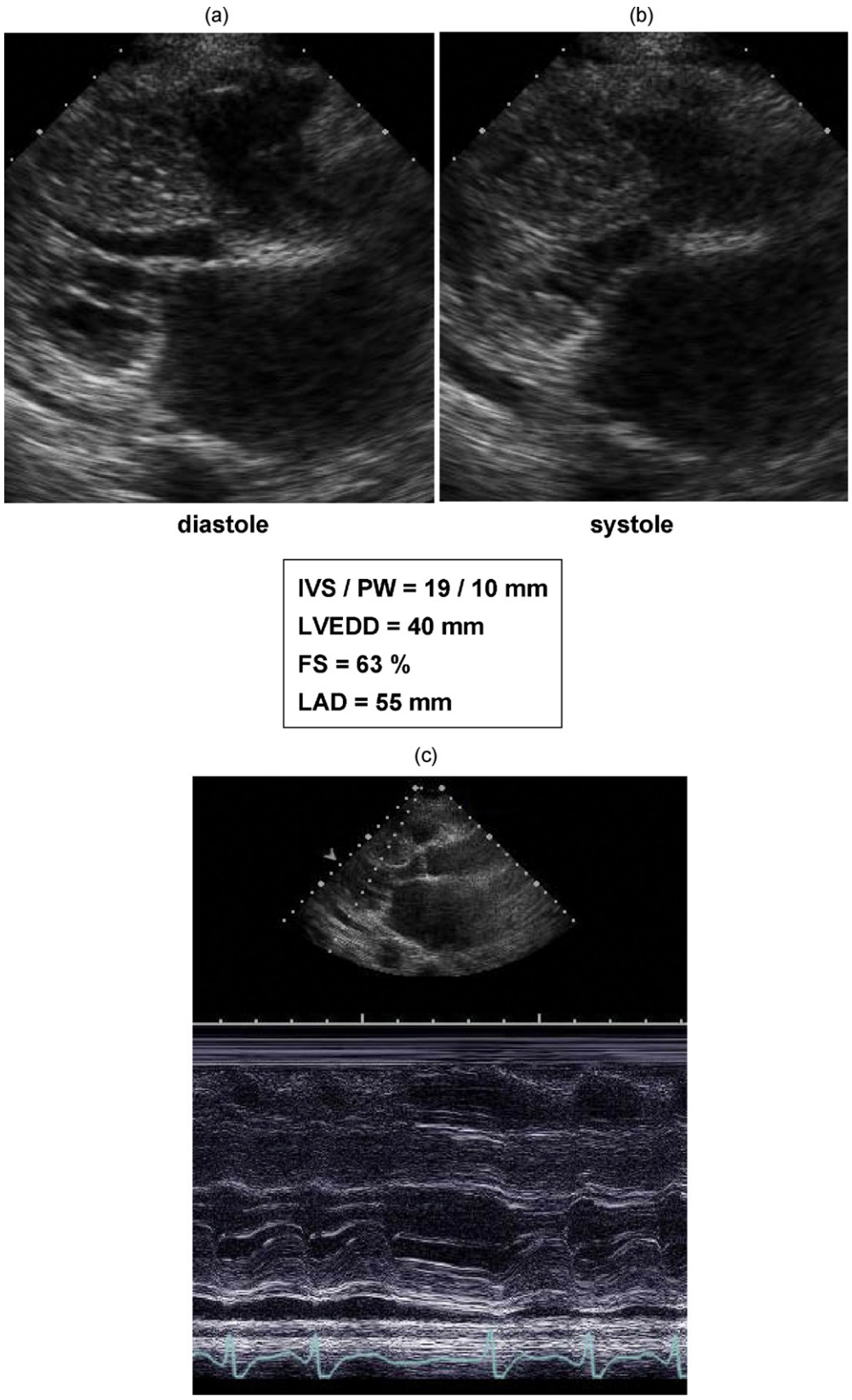

Figure 2 Images of transthoracic echocardiography. (a) 2D echocardiography (diastole) showing increased interventricular septal thickness of 19 mm and pericardial effusion. (b) 2D echocardiography (systole). (c) M-mode echocardiography showing normal systolic function. IVS, interventricular septum thickness; PW, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; FS, fractional shortening; LAD, left atrial diameter.

Table 1 Clinical characteristics of patients with an S297X mutation in *MYBPC3* at initial evaluation.

Patient no.	Gender	Age (years) at initial	Age (years) at diagnosis	Reason for diagnosis	NYHA class	Rhythm	Abnormal Q	ST-T change
H083-II-2	F	61	57	Symptom (chest pain)	I	Sinus rhythm	+	+
H083-III-2	F	40	38	Symptom (dyspnea)	II	Sinus rhythm	+	+
H083-III-4	F	39	35	ECG abnormality	I	Sinus rhythm	—	+
H083-IV-1	F	9	9	Family screening	I	Sinus rhythm	—	+
H083-IV-3	M	10	(10)	Family screening	I	Sinus rhythm	—	—
H087-II-6	F	75	75	Symptom (dyspnea)	II	Sinus rhythm	—	+
H087-III-2	F	58	58	Family screening	I	Sinus rhythm	—	+
H087-IV-1	M	36	36	Family screening	I	Sinus rhythm	—	+
H087-IV-3	M	32	32	Family screening	I	Sinus rhythm	—	—

MYBPC3, cardiac myosin-binding protein C gene; NYHA, New York Heart Association functional class; F, female; M, male; ECG, electrocardiography.

Table 2 Echocardiographic characteristics of patients with an S297X mutation in *MYBPC3* at initial evaluation.

Patient No.	IVS (mm)	PW (mm)	MLVWT (mm)	LVEDD (mm)	FS (%)	LAD (mm)	LVOTO	RVO	RVH	LVH pattern
H083-II-2	16	9	18	37	41	38	—	—	+	Maron III (base-apex)
H083-III-2	12	9	18	39	49	37	—	+	—	Maron III (mid-apex)
H083-III-4	21	9	26	38	51	39	+	—	+	Maron III (base-apex)
H083-IV-1	12	6	19	33	54	29	—	—	—	Concentric (mid-apex)
H083-IV-3	6	6	9	34	38	25	—	—	—	No LVH
H087-II-6	21	10	22	40	40	51	+	—	+	Maron III (base-apex)
H087-III-2	10	10	11	41	46	38	—	—	—	No LVH
H087-IV-1	9	9	16	54	42	43	—	—	—	Inferoseptum (mid-apex)
H087-IV-3	12	11	15	46	46	41	—	—	—	Anterolateral (base)

MYBPC3, cardiac myosin-binding protein C gene; IVS, interventricular septum thickness; PW, posterior wall thickness; LVEDD, left ventricular end-diastolic diameter; FS, fractional shortening; LAD, left atrial diameter; LVOTO, left ventricular outflow tract obstruction; RVO, intra-right-ventricular obstruction; RVH, right ventricular hypertrophy; LVH, left ventricular hypertrophy.

Table 3 Clinical course of patients with an S297X mutation in *MYBPC3* during follow-up.

Patient no.	Gender	Age (years) at initial	Rhythm change	NYHA change	Hospitalization for heart failure, age (years)	Status, age (years)
H083-II-2	F	61	SR → SR	I → I	–	Alive (stable), 66
H083-III-2	F	40	SR → SR	II → II	–	Alive (stable), 45
H083-III-4	F	39	SR → SR	I → I	–	Alive (stable), 43
H083-IV-1	F	9	SR → SR	I → I	–	Alive (developing LVH; 19 → 25 mm), 13
H083-IV-3	M	10	SR → SR	I → I	–	Alive (no LVH), 12
H087-II-6	F	75	SR → PAF	II → III	+, 86	Alive, 88
H087-III-2	F	58	SR → SR	I → I	–	Alive (no LVH on echocardiography), 60
H087-IV-1	M	36	SR → SR	I → I	–	Alive (stable), 37
H087-IV-3	M	32	SR → SR	I → I	–	Alive (stable), 33

MYBPC3, cardiac myosin-binding protein C gene; NYHA, New York Heart Association functional class; F, female; M, male; SR, sinus rhythm; PAF, paroxysmal atrial fibrillation; LVH, left ventricular hypertrophy.

having the disease at 9 years of age. On the other hand, another patient (H087-II-6) was diagnosed at the age of 75 years, although it was unknown when the patient actually developed hypertrophy. Although previous studies showed that mutations in *MYBPC3* were associated with delayed expression of hypertrophy, the present study indicates that early onset of HCM can be frequently seen in subjects with a *MYBPC3* mutation [12—16].

The distribution of hypertrophy was various, and maximum LV wall thickness ranged from 9 to 26 mm. From the point of hemodynamic findings, there were different subtypes of HCM: two patients had LV outflow tract obstruction and one patient showed intra-right-ventricular obstruction. The fact that patients with identical mutations showed heterogeneous clinical presentations suggests that other genetic and/or environmental factors are involved.

In the present study, the clinical course of this mutation seemed to be benign, although one patient was repeatedly hospitalized for the treatment of heart failure after the onset of atrial fibrillation at the age of 86 years. It is suggested that younger patients with this mutation must be followed carefully for a long period. We recently reported that a different truncation mutation in *MYBPC3* (V593fs: we altered the name V592fs/8) was associated with 'end-stage' HCM characterized by LV systolic dysfunction, cavity dilatation, and irreversible heart failure and speculated that a collapse of sarcomere stability compensated by residual protein in heterozygous patients might occur with advancing age and might lead to impaired contractile function in the elderly [20,24]. However, patients with S297X truncation mutation, even elderly patients, did not show LV systolic dysfunction, although the number of the patients with this mutation was small. On the other hand, Konno et al. reported that a missense mutation (R820Q) in *MYBPC3* is responsible for HCM with LV systolic dysfunction and dilatation [25]. Although truncation mutations are generally thought to cause greater alterations of protein structure and function than missense mutations do, these results indicate that truncation mutations do not always cause a more severe phenotype of the disease than do missense mutations from the clinical point of view. The exact biophysical properties altered by these abnormalities remain unknown. Further investigations of genotype—phenotype correlations are needed to clarify the pathogenesis of LV remodeling in HCM.

In conclusion, a novel mutation, S297X, in *MYBPC3* was identified in two of 93 probands with HCM. This nonsense mutation causes HCM in a broad range of ages and heterogeneous clinical manifestations, although the clinical course in patients with this mutation seems to be benign.

Conflict of interest

None.

References

[1] Spirito P, Seidman CE, McKenna WJ, Maron BJ. The management of hypertrophic cardiomyopathy. N Engl J Med 1997;336:775—85.

[2] Maron BJ, McKenna WJ, Danielson GK, Kappenberger LJ, Kuhn HJ, Seidman CE, Shah PM, Spencer 3rd WH, Spirito P, Ten Cate FJ, Wigle ED. Task force on Clinical Expert Consensus Documents. American College of Cardiology; Committee for Practice Guidelines. European Society of Cardiology. American College of Cardiology/European Society of Cardiology clinical expert consensus document on hypertrophic cardiomyopathy. A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. J Am Coll Cardiol 2003;42:1687—713.
[3] Maron BJ. Hypertrophic cardiomyopathy: a systematic review. JAMA 2002;287:1308—20.
[4] Elliott P, McKenna WJ. Hypertrophic cardiomyopathy. Lancet 2004;363:1881—91.
[5] Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy: from mutations to functional defects. Circ Res 1998;83:580—93.
[6] Arad M, Seidman JG, Seidman CE. Phenotypic diversity in hypertrophic cardiomyopathy. Hum Mol Genet 2002;11:2499—506.
[7] Fujino N, Shimizu M, Ino H, Okeie K, Yamaguchi M, Yasuda T, Kokado H, Mabuchi H. Cardiac troponin T Arg92Trp mutation and progression from hypertrophic to dilated cardiomyopathy. Clin Cardiol 2001;24:397—402.
[8] Watkins H, Rosenzweig A, Hwang DS, Levi T, McKenna W, Seidman CE, Seidman JG. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. N Engl J Med 1992;326:1108—14.
[9] Epstein ND, Cohn GM, Cyran F, Fananapazir L. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the beta-myosin heavy chain gene. Circulation 1992;86:345—52.
[10] Watkins H, McKenna WJ, Thierfelder L, Suk HJ, Anan R, O'Donoghue A, Spirito P, Matsumori A, Moravec CS, Seidman JG, Seidman CE. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. N Engl J Med 1995;332:1058—64.
[11] Moolman JC, Corfield VA, Posen B, Ngumbela K, Seidman C, Brink PA, Watkins H. Sudden death due to troponin T mutations. J Am Coll Cardiol 1997;29:549—55.
[12] Watkins H, Conner D, Thierfelder L, Jarcho JA, MacRae C, McKenna WJ, Maron BJ, Seidman JG, Seidman CE. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. Nat Genet 1995;11:434—7.
[13] Bonne G, Carrier L, Bercovici J, Cruaud C, Richard P, Hainque B, Gautel M, Labeit S, James M, Beckmann J, Weissenbach J, Vosberg HP, Fiszman M, Komajda M, Schwartz K. Cardiac myosin binding protein-C gene splice acceptor site mutation is associated with familial hypertrophic cardiomyopathy. Nat Genet 1995;11:438—40.
[14] Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, McKenna W, Kristinsson A, Roberts R, Sole M, Maron BJ, Seidman JG, Seidman CE. Mutation in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy. N Engl J Med 1998;338:1248—57.
[15] Maron BJ, Niimura H, Casey SA, Soper MK, Wright GB, Seidman JG, Seidman CE. Development of left ventricular hypertrophy in adults with hypertrophic cardiomyopathy caused by cardiac myosin-binding protein C gene mutations. J Am Coll Cardiol 2001;38:315—21.
[16] Anan R, Niimura H, Takenaka T, Hamasaki S, Tei C. Mutations in the genes for sarcomeric proteins in Japanese patients with onset sporadic hypertrophic cardiomyopathy after age 40 years. Am J Cardiol 2007;99:1750—4.
[17] Shapiro LM, McKenna WJ. Distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy: a two-dimensional echocardiographic study. J Am Coll Cardiol 1983;2: 437—44.
[18] Maron BJ, Gottdiener JS, Epstein SE. Patterns and significance of distribution of left ventricular hypertrophy in hypertrophic cardiomyopathy. A wide angle, two dimensional echocardiographic study of 125 patients. Am J Cardiol 1981;48:418—28.
[19] Hirota T, Kitaoka H, Kubo T, Okawa M, Furuno T, Doi YL. Morphologic characteristics of hypertrophic cardiomyopathy of the elderly with cardiac myosin-binding protein C gene mutations. Circ J 2006;70:875—9.
[20] Kubo T, Kitaoka H, Okawa M, Matsumura Y, Hitomi N, Yamasaki N, Furuno T, Takata J, Nishinaga M, Kimura A, Doi YL. Lifelong left ventricular remodeling of hypertrophic cardiomyopathy caused by a founder frameshift deletion mutation in the cardiac myosin-binding protein C gene among Japanese. J Am Coll Cardiol 2005;46:1737—43.
[21] Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation 2003;107:2227—32.
[22] Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, Ackerman MJ. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol 2004;44:1903—10.
[23] Flashman E, Redwood C, Moolman-Smook J, Watkins H. Cardiac myosin binding protein C: its role in physiology and disease. Circ Res 2004;94:1279—89.
[24] Hayato K, Okawa M, Matsumura Y, Kitaoka H, Kubo T, Hitomi N, Yamasaki N, Yabe T, Furuno T, Takata J, Nishinaga M, Doi YL. Hypertrophic cardiomyopathy with mild left ventricular remodeling: echocardiographic assessment using left ventricular wall motion score. J Cardiol 2008;51:95—105.
[25] Konno T, Shimizu M, Ino H, Matsuyama T, Yamaguchi M, Terai H, Hayashi K, Mabuchi T, Kiyama M, Sakata K, Hayashi T, Inoue M, Kaneda T, Mabuchi H. A novel missense mutation in the myosin binding protein-C gene is responsible for hypertrophic cardiomyopathy with left ventricular dysfunction and dilation in elderly patients. J Am Coll Cardiol 2003;41:781—6.